

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **10/816,081**
Applicants : **David B. Rozema et al.**
Filed : **04/01/2004**
Art Unit : **1636**
Examiner : **Dunston, Jennifer Ann**
Docket No. : **Mirus.035.02.1**
For: **Endosomolytic Polymers**

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Dr. David B. Rozema, hereby declare as follows:

1. I have a Doctorate in Chemistry from the University of Wisconsin, Madison.
2. I am familiar with the above captioned application.
3. I submit, with this Response, experimental material illustrating the ability of polyethyleneimine to cause liposome leakage.

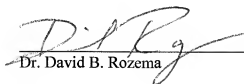
Liposomes composed of DPPS:DOPE:DOPC (dipalmitoyl phosphatidyl serine : dioleoyl phosphatidyl ethanolamine : dioleoyl phosphatidyl choline) at 15:25:60 molar ratio were formulated in HEPES buffered saline (HBS) containing 100 mM calcein at pH 7.5. The unencapsulated calcein was removed by size exclusion chromatography. The liposomes were then added to a solution of HBS followed by addition of various amounts of either branched polyethyleneimine 25kDa molecular weight (PEI), cationic amphipathic polyvinylether composed of amino, butyl, and octadecyl groups (polymer 1360), or Triton X-100 (detergent commonly used to permeabilize cell membranes and known to disrupt liposomes). 30 minutes after addition of polymers or triton, the fluorescence of calcein was measured: $\lambda_{\text{excitation}}=495$, $\lambda_{\text{emission}}=520$. The percentage of liposome lysis was determined as follows:

$$\frac{\text{fluorescence (liposome + polymer)} - \text{fluorescence (liposome)}}{\text{fluorescence (liposomes + 100 } \mu\text{g Triton X-100)} - \text{fluorescence (liposome)}}$$

polymer	% lysis
PEI; 1 $\mu\text{g/mL}$	0
PEI; 40 $\mu\text{g/mL}$	0
PEI; 800 $\mu\text{g/mL}$	0
amphipathic polyvinylether; 1 μg	87
Triton X-100	100

The results show that PEI did not cause a detectable level of leakage of calcein from the liposomes.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

 4/25/08
 Dr. David B. Rozema date